2.Antimetabolites

Most antimetabolites are effective cancer chemotherapeutic agents via interaction with the biosynthesis of nucleic acids.

Therefore, several of the useful drugs used in antimetabolite therapy are purines, pyrimidines, folates, and related compounds.

The antimetabolite drugs may exert their effects by several individual mechanisms involving enzyme inhibition at active, allosteric, or related sites. The purine and pyrimidine antimetabolites are often compounds incorporated into nucleic acids and the nucleic acid polymers (DNA, RNA, etc.).

The antifolates are compounds designed to interact at cofactor sites for enzymes involved in the biosynthesis of nucleic acid bases.

A. Pyrimidine Drugs (uracil derivatives)

The pyrimidine derivative 5-fluorouracil (5-FU) was designed to block the conversion of uridine to thymidine.

The normal biosynthesis of thymidine involves methylation of the 5-position of the pyrimidine ring of uridine.

The replacement of the hydrogen at the 5-position of uracil with a fluorine results in an antimetabolite drug, leading to the formation of a stable covalent ternary complex composed of 5-FU, thymidylate synthase (TS), and cofactor (a tetrahydrofolate species).

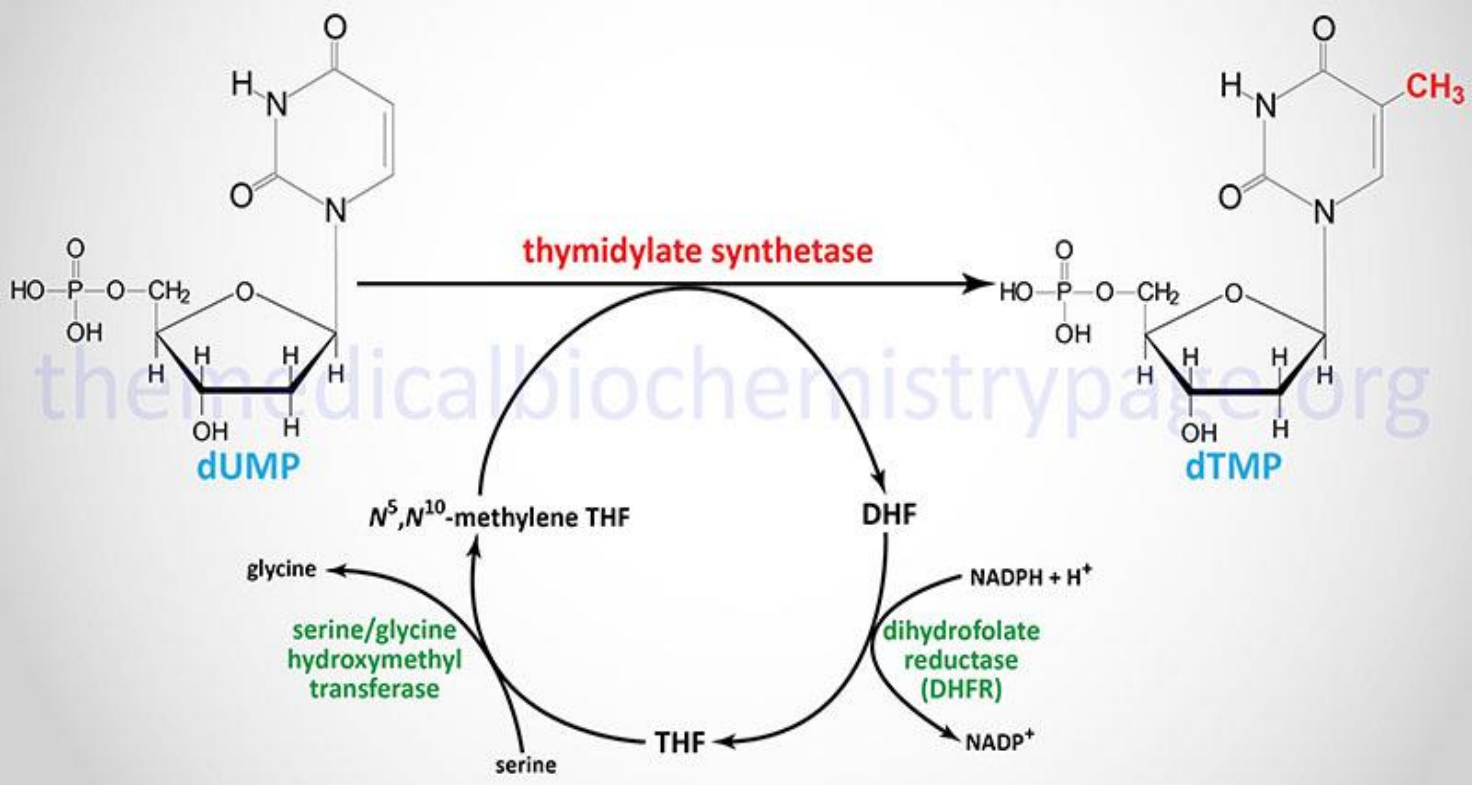
The metabolic activation (anabolism) of 5-FU required to produce the anticancer effects accounts for no more than 20% of the administered amount of drug in most patients.

- Catabolic inactivation via the normal pathways for uracil consumes the remaining approximate 80% of the dose. The major enzyme of pyrimidine catabolism is dihydropyrimidine dehydrogenase (DPD), and 5-FU is a substrate for this enzyme.
- Uracil is a substrate for this enzyme system also and has been dosed with 5-FU and 5-FU prodrugs in an attempt to saturate DPD and conserve active drug species.
- Variability in the levels of DPD activity among the patient population is a major factor in the bioavailability of 5-FU.

- low bioavailability of 5-FU as a result of the catabolic efficiency of DPD and other enzymes has lead to the development of unique dosing routes and schedules as well as the development of prodrug forms of 5-FU.
- TS is responsible for the reductive methylation of deoxyuridine monophosphate (dUMP) by 5,10-methylenetetrahydrofolate to yield dTMP and dihydrofolate.
- Because thymine is unique to DNA, the TS enzyme system plays an important role in replication and cell division.
- The tetrahydrofolate cofactor species serves as both the one-carbon donor and the hydride source in this system.

Biosynthesis of Thymidine

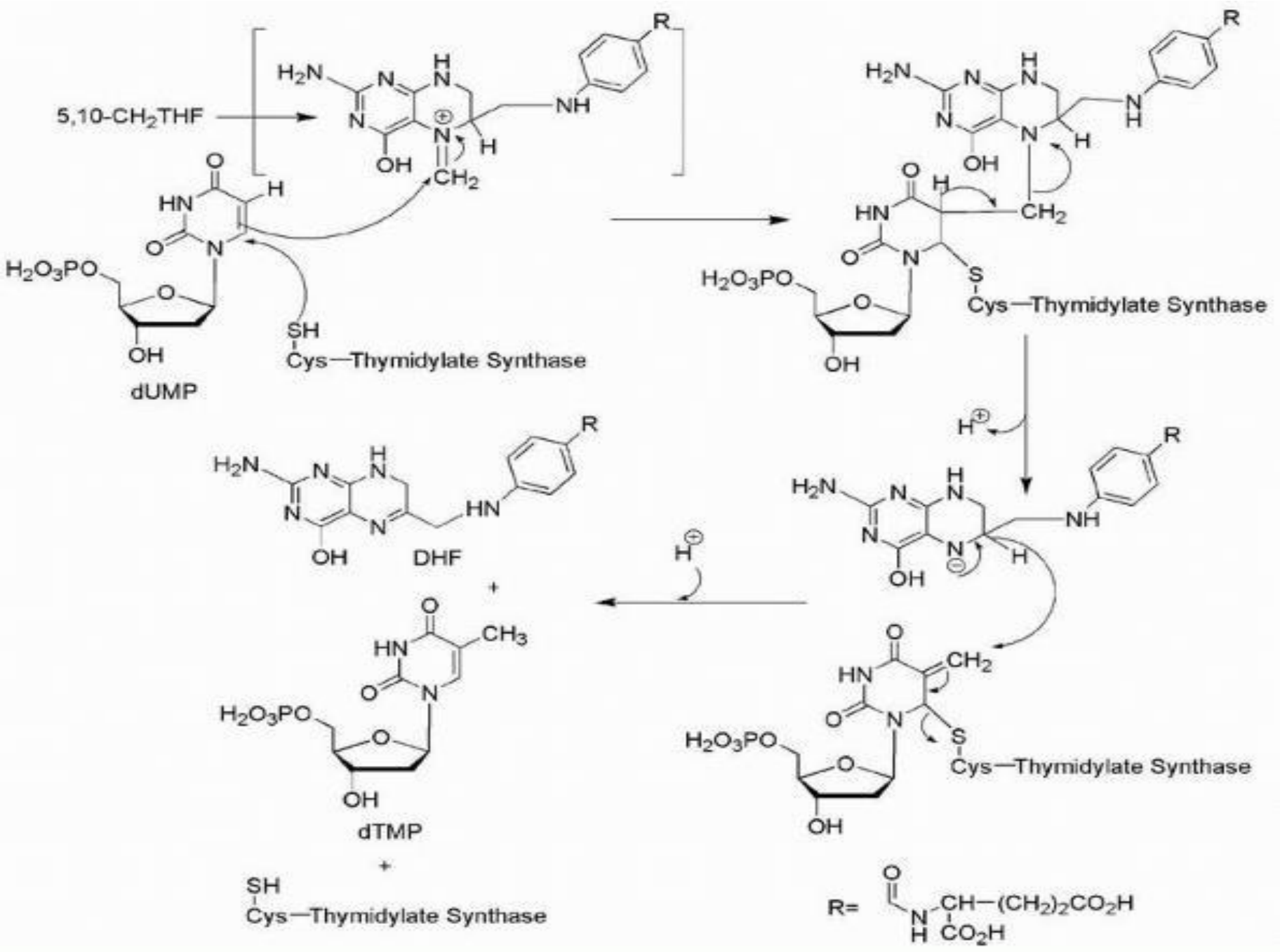
The normal biosynthesis of thymidine involves methylation of the 5-position of the pyrimidine ring of uridine.



- The initial step of the process involves the nucleophilic attack by sulfhydryl group of a cystine residue at the 6-position of dUMP.
- The resulting enolate adds to the methylene of 5,10- CH2-THF perhaps activated via the very reactive N-5- iminium ion .
- The iminium ion likely forms at N-5 and only after 5,10-CH2-THF binds to TS.

The iminium ion is likely formed at N-5 because it is the more basic of the two nitrogens, whereas N-10 is the better leaving group.

- The loss of the proton at the 5-position of dUMP and elimination of folate yields the exocyclic methylene uracil species.
- The final step involves hydride transfer from THF and elimination to yield the enzyme, DHF, and dTMP.

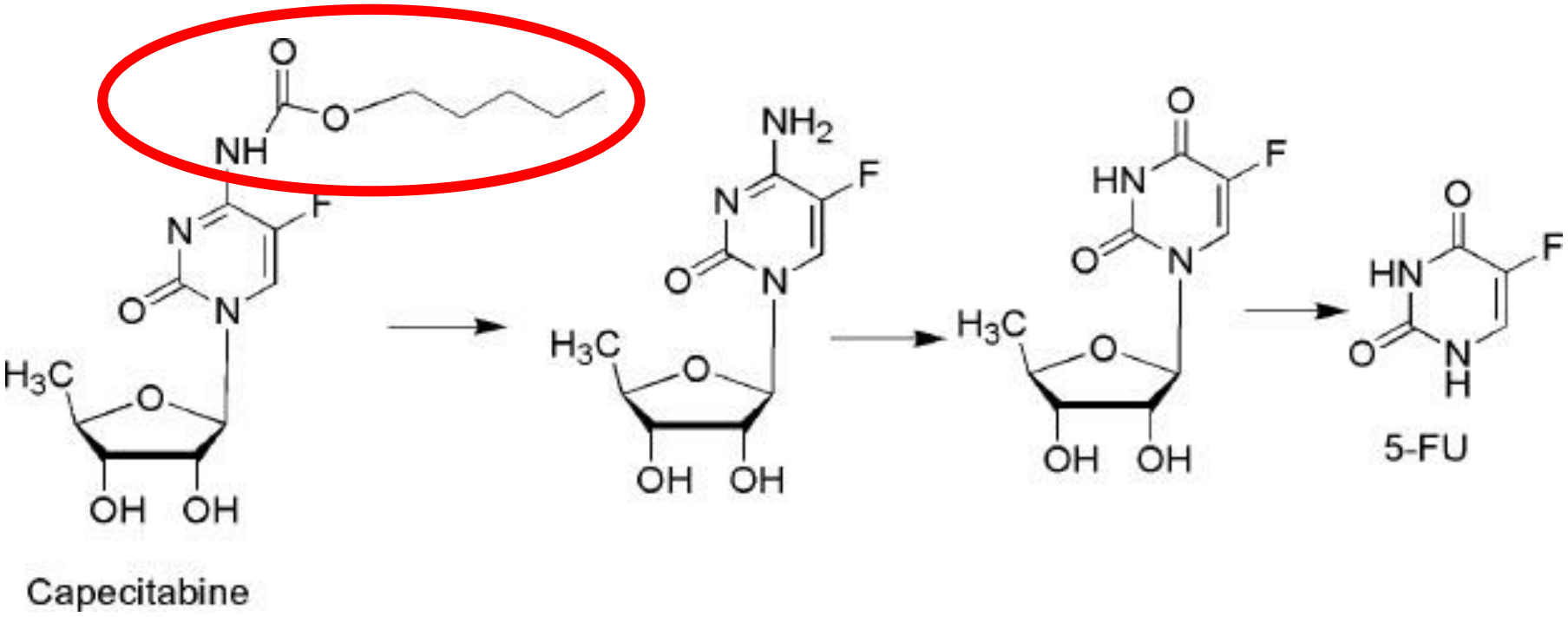

5,10-CH2THF
dUMP
SH
Cys—Thymidylate Synthase
Cys—Thymidylate Synthase
H+
DHF
+
dTMP
+
SH
Cys—Thymidylate Synthase
Cys—Thymidylate Synthase
R=

Biochemical conversion of uridine to thymidine.

- Attempts at chemical modification of 5-FU to protect from catabolic events have produced several prodrug forms, which are converted via in vivo metabolic and/or chemical transformation to the parent drug 5-FU.
- The carbamate derivative of 5-deoxy-5-fluorocytidine is known as capecitabine, and it is converted to 5-FU through a series of activation steps.
- The tetrahydrofuran derivative tegafur is slowly converted to 5-FU but requires quite high doses to reach therapeutic plasma concentrations.
- Esters of the N-hydroxymethyl derivative of tegafur show greater anticancer activity than tegafur.

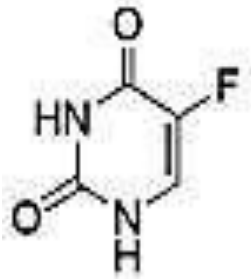
Metabolic activation of capecitabine to 5-FU

1. Carbamate hydrolysis (decarbamylation).
2. Deamination.
3. Hydrolysis of the sugar moiety to yield 5-FU.

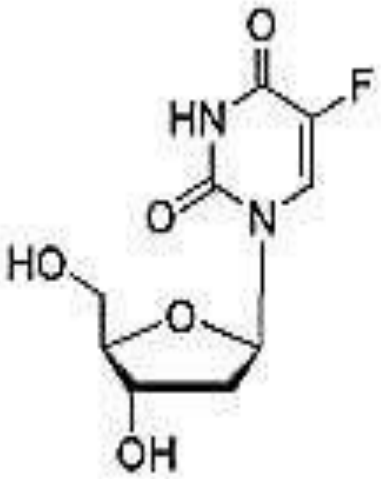


Capecitabine

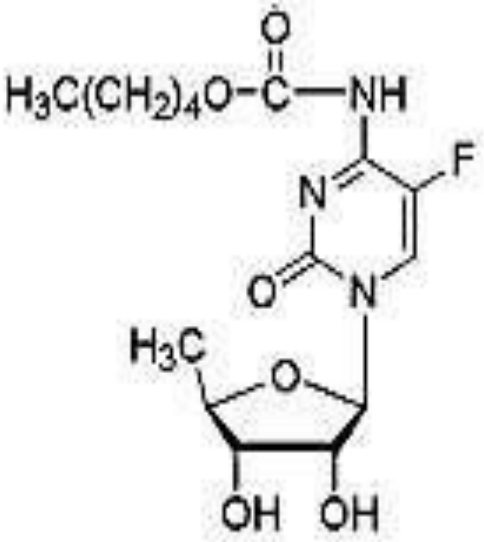
Pyrimidine Drugs



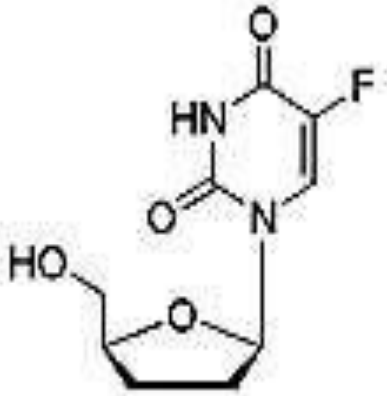
5-Fluorouracil



Floxuridine



Capecitabine



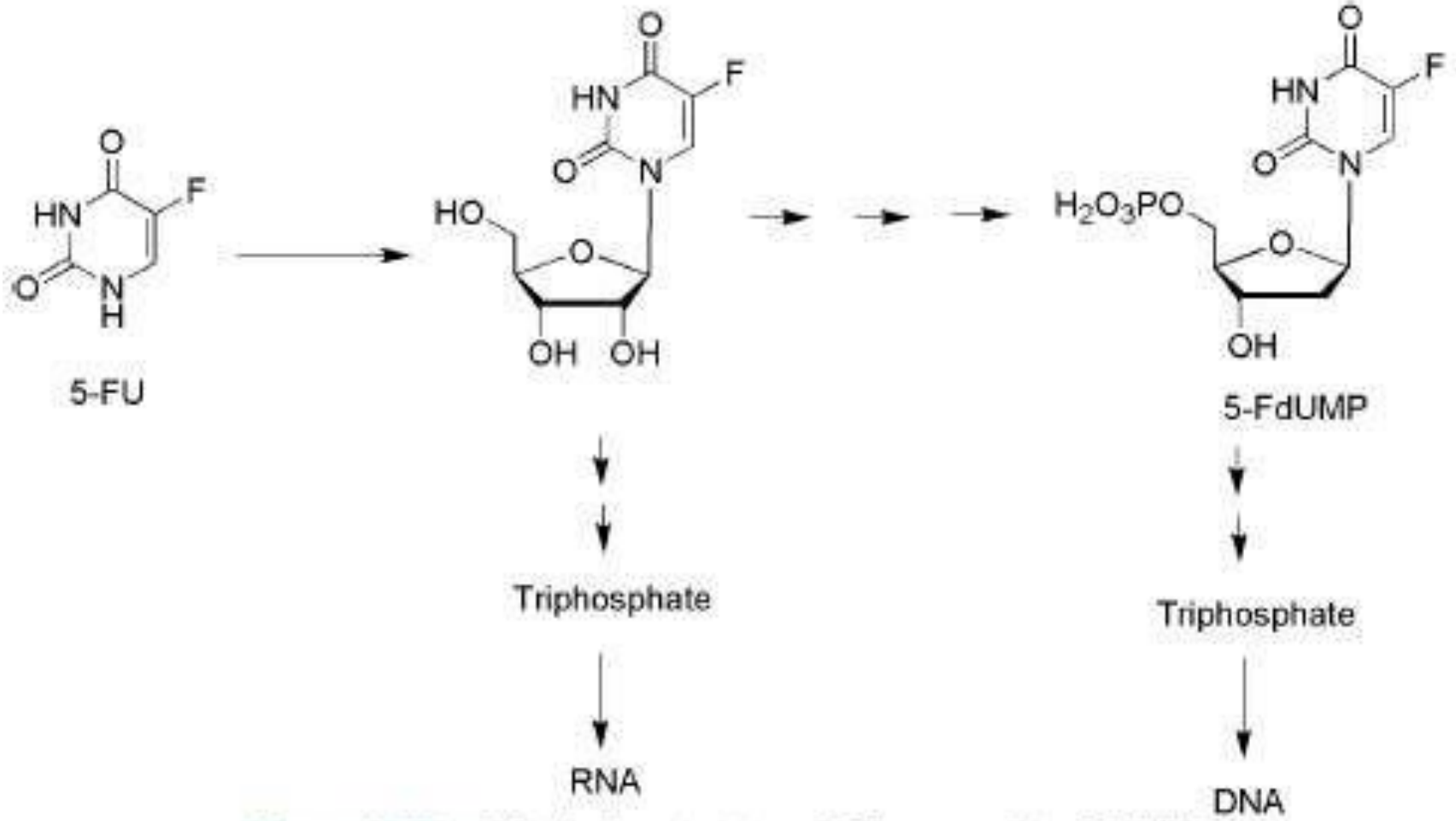
Tegafur

5-Fluorouracil is activated by conversion to the corresponding nucleotide species, 5-fluoro-2-deoxyuridylic acid.

The resulting 5-fluoro-2-deoxyuridylic acid is a powerful inhibitor of thymidylate synthetase , the enzyme that converts 2-deoxyuridylic acid to thymidylic acid.

In the inhibiting reaction, the sulfhydryl group of TS adds via conjugate addition to the 6-position of the fluorouracil moiety.

The carbon at the 5-position then binds to the methylene group of 5,10-Methylene tetrahydrofolate following initial formation of the more electrophilic form of folate the N-5-iminium ion.

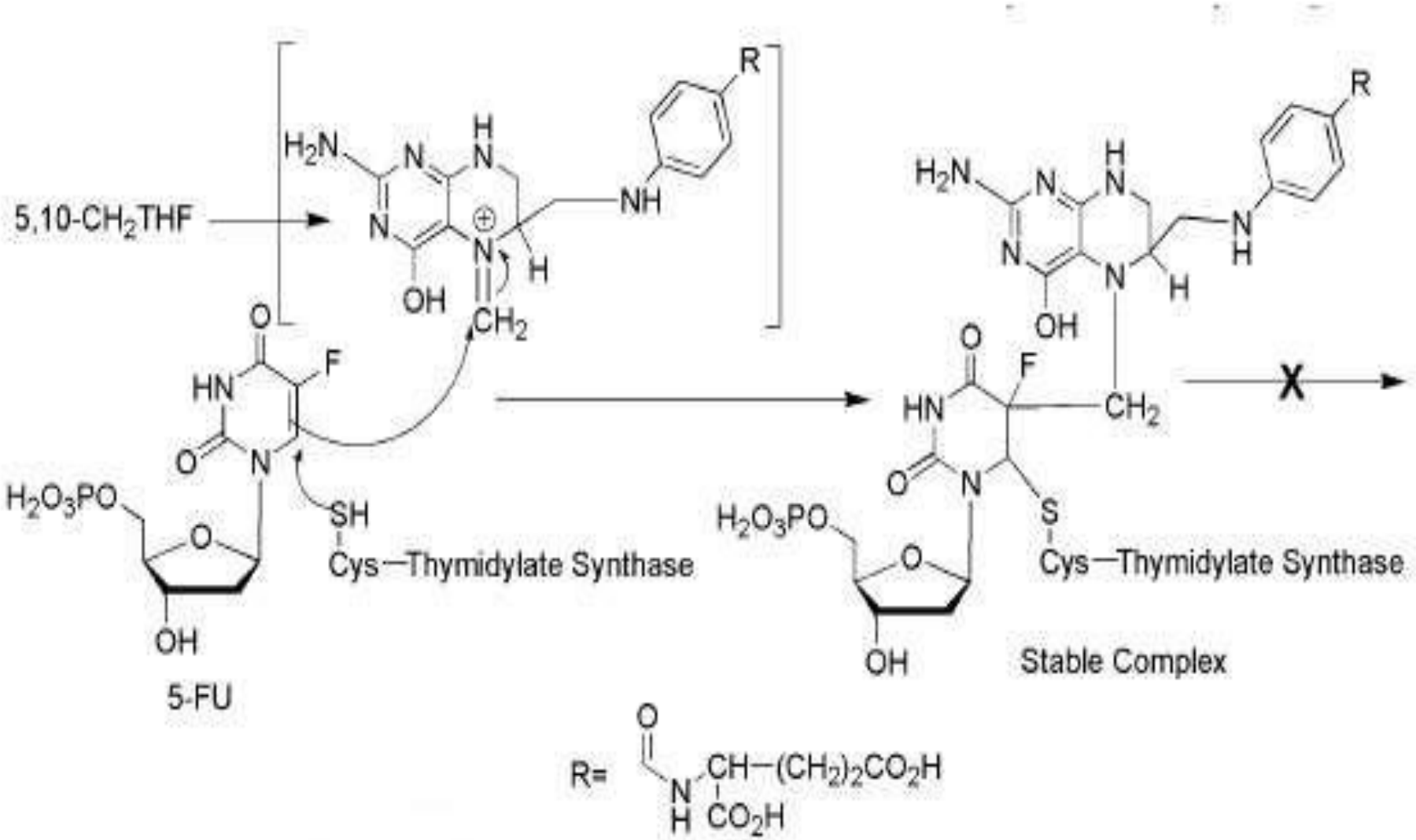


Scheme 10.18 • Metabolic activation of 5-fluorouracil to 5-FdUMP.

In the normal process, this step is followed by the elimination of dihydrofolate from the ternary complex, regeneration of the active enzyme species, and the product thymidine.

Central to this process is the loss of the proton at the 5-position of uracil to form the exocyclic methylene uracil species.

The 5-fluorine is stable to elimination, and a terminal product results, involving the enzyme, cofactor, and substrate, all covalently bonded.



Scheme 10.19 • Mechanism of inhibition of TS by 5-fluorouracil.

Cytarabine and gemcitabine(cytosine derivatives)

Pyrimidine analogs as antimetabolites for cancer therapy have been developed based on the cytosine structure as well.

Modification of the normal ribose or deoxyribose moiety has produced useful drug species such as cytarabine (ara-C) and gemcitabine. Cytosine arabinoside (ara-C or cytarabine) is simply the arabinose sugar instead of ribose, and the only difference in structure is the epimeric hydroxyl group at the 2-position of the pentose sugar.

Mechanism of action may include a slowing of the DNA chain elongation reaction via DNA polymerase or cellular inefficiencies in DNA processing or repair after incorporation.

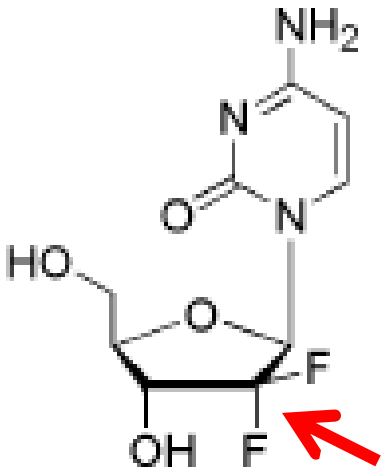
Gemcitabine is the result of fluorination of the 2`- position of the sugar moiety. After its anabolism to diphosphate and triphosphate metabolites, it inhibits ribonucleotide reductase and competes with 2-deoxycytidine triphosphate for incorporation into DNA.

The mechanism of action for gemcitabine is likely similar to that of ara-C including alteration of the rate of incorporation into DNA as well as the rate of DNA processing and repair.

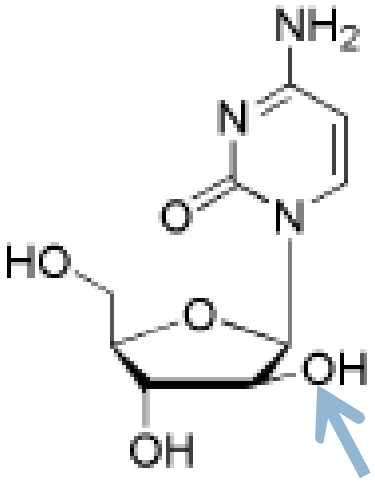
Modification of the pyrimidine ring has also been explored for the development of potential anticancer drugs based on antimetabolite theory.

Several pyrimidine nucleoside analogs have one more or one less nitrogen in the heterocyclic ring. They are known as azapyrimidine or deazapyrimidine nucleosides. 5-Azacytidine is an example of a drug in this category.

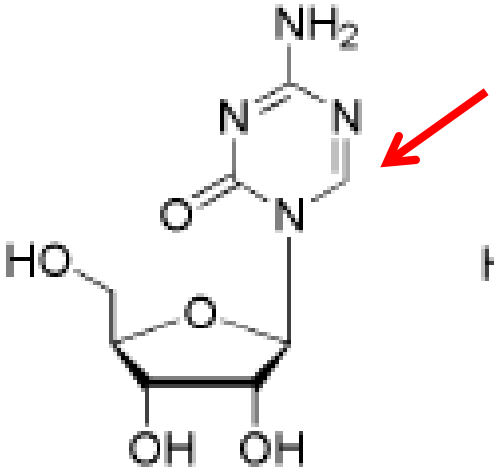
Anticancer drugs based on pyrimidine and related compounds



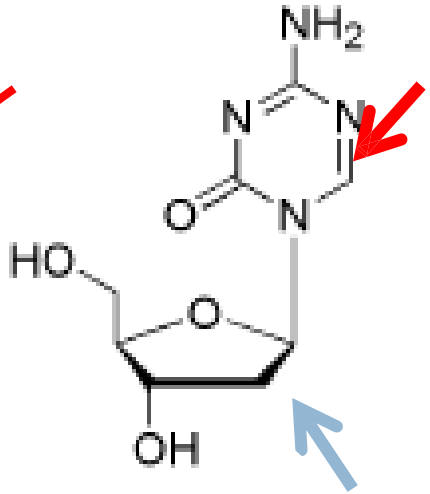
Gemcitabine



Cytarabine



5-Azacytidine



Decitabine

The mode of action of this compound is complex involving reversible inhibition of DNA methyl transferase, and this lack of methylated DNA activates tumor suppressor genes. In certain tumor systems, it is incorporated into nucleic acids, which may result in misreading or processing errors.